Studies on taxonomy of spider fauna (Arachnida: Araneae) in the Himalayan region of Kashmir

PhD Synopsis

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INTRODUCTION

Spiders are cosmopolitan in distribution and are included in Phylum Arthropoda, class Arachnida and order Araneae. Class Arachnida includes arthropods that have two body regions, one is the cephalothorax, which bears four pairs of legs and other is the abdomen. Araneae forms the largest order of Arachnida, comprising of spiders as the 7th most diverse group of organisms in the world. Spiders are different from other Arachnids because of the presence of 2 structures, one is Pedicle (a constricted stem like structure that joins the cephalothorax with the abdomen) and other is the presence of spinneret at the hind region of abdomen which acts as an organ to release silk in spiders. The origin of spiders is monophyletic i.e. they are a clade of organisms comprising of all descendants with their common ancestor (Shultz, 2007).

Spiders are very important as far as their ecological role in food webs is considered; being natural predators they have been applied in IPM (Integrated Pest Management) to control insect pests of horticultural and agricultural ecosystems (Michalko *et al*., 2018). Various disease causing mosquitoes have also been controlled by using spiders (Ndava *et al*., 2018). Spiders have been canvassed as prototype organisms for behavioural (George *et al*., 2016) and ecological (Pruitt & Riechert, 2012) studies. Developmental (Hilbrant *et al.*, 2012) and evolutionary (Mc Gregor *et al*., 2008) studies on spiders have provided insights for other organisms too. Spiders are also known to act as indicator species for environmental monitoring (Ossamy *et al.*, 2016). Spider silk has various applications in biological, chemical, biomedical, engineering and defense areas (Vendrely & Scheibel, 2007).

Taxonomy of Spiders is an under-researched field, however it is promptly flourishing and numerous new species are being reported each year. Globally species richness of spiders could be no less than 120,000 (Agnarsson *et al.*, 2013); therefore, a huge percentage of spider diversity lives undiscovered. Identifications on the basis of morphology of spiders is strenuous which is attributable to the absence of keys for identification of juveniles, sexual dimorphism and polymorphism found in this group. (Coddington & Levi, 1991; Magalhaes *et al.*, 2017).

Taking into account these impediments, it becomes necessary to use supplementary techniques for expeditious and accurate species identification. DNA barcoding is a technique that utilizes short and standardized portions of genes for species identification (Hebert *et al*., 2003). DNA barcoding has an immense potential for swift and correct taxonomic resolutions. It has been widely

used in taxonomic research for resolving taxonomic questions (Laskar *et al.,* 2018), identification of cryptic species and species complexes (Tyagi *et al.*, 2017), accurate delimitation and identification of species (Hebert *et al.*, 2003) and population estimation (Khalili *et al.,* 2016). A 648 bp DNA barcode region in mitochondrial COI gene is good for identification of species as it has great degree of discrimination among animal groups. Identification of species through DNA barcoding of mtCOI (mitochondrial cytochrome oxidase gene I) is an efficient approach for species identification (Hebert *et al*., 2003).

REVIEW OF LITERATURE

I. Review of important world literature on spiders:

Spider taxonomy has been studied by World taxonomists since eighteenth century. The pioneers who prepared early taxonomic records on spiders were Latreille (1804) and Simon (1889). Spiders of Malaysia were studied by Workman (1896). Petrunkevitch (1933) studied internal anatomy of spiders. Locket and Millidge (1951) studied spiders of Great Britian. Spiders of Taiwan were studied by Lee (1966). Lehtinen (1967) studied comparative and phylogenetic system of classification of spiders. Australian spiders were documented by Mascord (1970). Kaston (1978) provided a summary of North American spiders. Brignoli (1983) provided a catalogue of 7000 species of spiders. Preston and Ken (1984) described the then known spiders of the world. Roberts (1985) studied spiders of Ireland and provided information about the morphology, behavior, classification, nomenclature, collection and preservation of spiders. He also devised a key to families of Ireland spiders. Davies & Zabka (1989) laid out key to Salticidae spider genera of Australia. Platnick (1989) came up with bibliography (1981-1987) of work relating to the taxonomy of Araneae. Checklist of Korean spiders was published by Kim (1991). Spiders of Madagascar were studied by Ono (1993). Vincent (1993) provided a field guide to spiders of North America. The field guide for spiders of North Europe and Britain was published by Roberts (1995). Spider catalogues were provided by Bonnet (1945, 1955-1959). Helsdingen (1996a, b) provided a catalogue on Irish spiders. Mikhailov (1997) provided a catalogue of spiders of former Soviet Union. Song & Zhu (1997) worked on spiders of China and published keys to species, genera and subfamilies of families Thomisidae and Philodromidae. Zabka & Kupryjanowicz (1997) worked on spiders of Poland and provided a key of 56 species of Salticidae family. Tanikawa (1998) studied Japanese spiders of genus *Neoscona*. Stirnaman *et al.* (1998) provided a checklist of 480 spider species of Missouri, USA. A checklist of 973 spider species from Romania was provided by Weiss & Petrison (1999). A checklist

of 645 spider species from Britain was provided by Merrette & Murphy (2000). Marusik *et al.* (2000) worked on spiders of Siberia and reported 96 species of spiders. Yoshida (2001) wrote a book on taxonomy of Japanese spiders. Deeleman (2001) wrote a book on forest spiders of Southeast Asia. Namkung (2001 & 2002) worked on Korean spiders. Shakila *et al.* (2002) worked on diversity and abundance of spiders in date palm from Pakistan. Levi (2002) studied spiders of America. Kunter & Sereg (2002) worked on spider fauna of Slovenia. Bonte *et al.* (2002) worked on the impact of plant community, ecosystem and abiotic factors on spider assemblages. Zhu *et al.* (2003) wrote a book describing family Tetragnathidae. Namkung (2003) wrote a book on Spiders of Korea. Spiders of Vietnam were studied by Tu and Li (2004). World Spider Catalogue was launched on internet by Platnick (2004). Churchill and Ludwig (2004) worked on spider assemblages in grasslands of Australia. Molur & Siliwal (2004) worked on spiders of South East Asia. Kok *et al.* (2004) and Orchinnikov & Inayatuallah (2005) worked on spiders of Pakistan. Zschokke & Herberstein (2005) came up with a guide on how to collect, keep and study spider webs. They also explained how to encourage spiders to build webs. Jocque *et al.* (2005) quantified spider density in Ivory Coast rain forests. Dunlop *et al.* (2008) reported fossil species of Arachnids. Jaime & John (2008) worked on performance of pit fall traps in collecting spiders. Corcuera *et al*. (2008) calculated the effect of micro architecture of Mexican dry forest foliage on spider distribution. Spiders have been identified all across the globe using DNA barcoding. Spiders of Canada were identified using DNA barcoding (Robinson *et al.*, 2009; Blagoev *et al.*, 2013 and Blagoev, 2016). Gray (2010) studied Australian funnel web spiders. Carvalho *et al.* (2011) reported that distribution of spider fauna is highly sensitive to habitat structure. Platnick (2011) updated the World Spider Catalog. Farzana *et al.* (2012) studied spider diversity of Peshawar, Pakistan. Chetia & Kalita (2012) reported that spider populations decline as weather changes from summer to winter. Kashefi *et al.* (2013) studied spider fauna of Iran. Platnick (2013) updated the World Spider Catalog. Huber *et al.* (2014) studied taxonomy of spiders of Venezuela. Shuqiang & Qingyuan (2014) studied spider fauna from Xishuangbanna tropical rain forests, China. Dzulhelmi *et al*. (2014) studied Malaysian spiders. Khan & Zaman (2015) made an attempt to study the spiders of Buner, Pakistan. Candek & Kuntner (2015) explored the usage of DNA barcoding in identification of species of spiders. World Spider catalogue (2015) was updated to its version 16.5. DNA barcodes for spiders of USA have been documented (Coddington *et al.*, 2016). DNA barcoding was used to identify spiders of Germany (Astrin *et al*. 2016). Naseem & Tahir (2016) used mtCOI gene for the identification of spiders of families Lycosidae and Salticidae of Pakistan. Viera & Gonzaga (2017) published a book entitled, 'Behaviour and Ecology of Spiders' which accounts for spiders found in Neo-Tropical regions of the world. The data from DNA barcoding has been used to study phylogeny of Araneae (Wheeler *et al.*, 2017). 35

new species of *Pimoa* genus from Pan-Himalayas with their DNA barcodes were reported by Xu *et al*. (2021). DNA barcoding of spiders of Finland was carried out by Roslin *et al*. (2021). World Spider Trait database has been launched in October 2021 and it is serving as an online database for recording spider traits worldwide (Pekar *et al.,* 2021). World Spider Catalog (2021), Version 22.0 which serves as an online database for spider taxonomy and describes 128 families of spiders containing 4208 genera and 49,180 species that have reported till date globally.

II. Review of Indian literature on spiders:

Out of the 49,180 species of spiders found worldwide 3.77% (1857 species) are found in India. Studies on Indian spiders were started in 19th century by some European workers. Introductory contributions were made by Stoliczka (1869) and Karsch (1873). Species from Himalayas were reported by Simon (1889). A catalogue of 200 species of Burmese spiders was published by Thorell (1895). Simon's work on spider fauna of Asian region (1889) provides useful information on spiders of Indian and Oriental region. Pocock (1900a) studied spiders of genus *Poecilotheria* from India. He presented first ever list with description of spiders of Ceylon, Burma and India in his book "Fauna of British India, Araneae" (1900b). He also presented reports on Oriental mygolomorphos (1895), and Indian arachnids (1899a, 1899b & 1901). He also worked on spiders of Lakshadweep islands (1904). Mimicry in Indian spiders was studied by Gravely (1912). Narayan (1915) studied ant-like spiders of family Salticidae in India. Gravely (1915) worked on Indian mygalomorph spiders. Pioneer work in South Indian arachnology was done by Sheriff (1919, 1927a, 1927b, 1927c). Gravely (1921a) studied spiders and scorpions of Barkuda Island, Chilka lake. Sub family Tetragnathinae and families Ctenidae, Sparassidae, Selenopidae and Clubionidae of Indian spiders were studied by Gravely (1921b, 1931). Spider fauna of Assam was described by Fage (1924). Relmoser (1934) also provided valuable information on Indian spiders. Indian spiders were also studied by Dayal (1935). Bhattacharya (1935a, b; 1941) worked on ant-like spiders and giant crab spider of India. Sinha (1951a, b) worked on Indian spiders of Lycosidae, Argiopidae, & Hersilidae. Sen & Basu (1963) worked on Indian spiders of Thomisidae. Patel (1973; 1975a, b, c; 1988a, b) described spider fauna of Gujarat. Tikader was the leading expert of Arachnology in India. He laid a firm foundation of Arachnology in India with his work. Tikader described spiders of Maharashtra (1963), spider fauna of Khasi and Jaintia hills (1966, 1968, 1969), and spiders of Sikkim (1970). Tikader (1976) provided a key to Indian spiders. Spiders of Andaman and Nicobar Islands were studied by Tikader (1977). Spiders of Maharashtra were studied by Tikader & Malhotra (1974 & 1980). Tikader (1980) wrote a book on Indian Thomisid spiders. Tikader & Biswas (1981) studied spiders of Bengal. Gajbe (1984, 1987, 1995a, 1995b, 1999, 2003) studied spiders of families Gnaphosidae and Oxyopidae, spider

fauna of Kanha tiger reserve and prepared a spider checklist for Chattisgarh and Madhya Pradesh. Biswas (1984a & b) studied spider families Araneidae, Clubionidae, Gnaphosidae and Salticidae. First comprehensive list of spider species of India was given by Tikader (1987). Sethi & Tikader (1988, 1990) worked on spiders of Heteropodidae in India. Patel & Reddy (1988) worked on spiders of Andhra Pradesh. Clubionid spiders were studied by Majumdar & Tikader (1991). A list of spiders of West Bengal was provided by Biswas & Biswas (1992). A book entitled 'Spiders: An Introduction' was written by Vijayalakshmi & Ahimaz (1993). Patel & Vyas (2001) described spiders from Andhra Pradesh. Amaurobiidae, Thomisidae and Salticidae spiders were studied by Bastawade (2002). Biswas & Biswas (2003) studied spiders of Sikkim. Biswas & Biswas (2004) studied spiders of North-east India from Manipur and West Bengal. Discovery of new species from South India was done by Smith (2004). Spiders of Agro-ecosystems were studied by Sebastain *et al.* (2001, 2004). Siliwal *et al.* (2005) provided a checklist of 1442 species of Indian spiders. Hazarika and Chakrabarti (2007) studied spiders of tea ecosystem in Assam. Sebastian & Peter (2009) provided information on Indian spiders in their book, 'Spiders of India'. Chakrabarti (2009) studied spiders of West Bengal. Biswas & Biswas (2010) worked on spiders of Uttarakhand. Sharma *et al.* (2010) worked on spider of Narmada river at Rajghat. Hippargi *et al.* (2011) gave account of spiders from Lonar, Melghat and southern tropical thorn forests of Solapur, Maharashtra. Keswani *et al*. (2012) provided a checklist of spiders of India with 60 families forming 438 genera including 1685 species. Wankhade *et al.* (2012) reported diversity and abundance of spiders at University of Pune. Chakrabarti (2013) published a pictoral field guide on Spiders of Western Himalayas. Patil & Uniyal (2013) studied spider dispersal. Keswani (2014) worked on diversity of spiders in citrus agroecosystems. Hadole *et al.* (2015) have worked on precinctive spider species of India. Dhali *et al.* (2016) worked on distribution and diversity of primitive spiders of India. DNA barcoding was employed to identify some spider species in Western Ghats (Gaikwad *et al.*, 2016). Malik & Goyal (2017) described biodiversity of spiders of Western Haryana. Rajpoot (2018) presented a molecular evidence of *Araniella cucurbitina*, a spider species from Uttarakhand. Tyagi *et al.* (2019) worked on DNA barcoding as a tool for identification of spiders of India. Sankaran *et al.* (2020) worked on taxonomic validity of Indian ground spiders. Smitha & Sudhikumar (2020) documented the diversity of spiders from cashew ecosystems of Kerela. 'Araneae of India' is an online data base that provides information about spiders of India. It is updated in accordance with World Spider Catalog. Presently, 1857 species categorised in 477 genera and 61 families are known in India.

III. Review of Literature from Kashmir:

Spider fauna of Kashmir valley has neither been explored out thoroughly, nor studied systematically. Very few records are available on the diversity of spiders of Kashmir. Except for the studies by Khan (2009 & 2011a, b) who reported 51 spider species from fruit orchards, rice fields and vegetable crops and Punjoo & Bhat (2015) who reported spiders from Dachigam National Park, Kashmir, no other work has been done. Therefore, due to lack of proper biodiversity studies on the spiders of Kashmir, there is a need for intensive and extensive surveys in this region. The present study would help in generating baseline data of this least studied groups of arthropods i.e. spiders of Kashmir.

Gap in research:

Many workers have undertaken the identification and taxonomy of spiders from India, but thorough survey and taxonomy of spiders of Himalayan region of Kashmir is yet to be undertaken. In India, a lion's share of the species have been reported and described from tropical areas and Kashmir falls under temperate area, thus different fauna is expected here. Therefore, exploration, identification and taxonomic work on spider fauna is to be carried out thoroughly and systematically from the areas covering Himalayan region of Kashmir. Moreover, no work has been done pertaining to identification in detail, systematic exploration, arrangement of available species in keys to genera and keys to species among genera. Study of genitalia for species confirmation has been carried out by few workers in India, it should also be undertaken and to be studied thoroughly from this region.

RATIONALE OF STUDY

The available information about spiders of Himalayan region of Kashmir is scanty in comparison to other regions of India. As such, there is a genuine requirement to explore the spider diversity in the said part of India. Also, no key to genera and species of spiders from Himalayan region of Kashmir is available till date. The proposed study will help in correct identification and systematic taxonomic description of spider fauna, as well as devise keys to genera and species of spiders from Himalayan region of Kashmir. This will be the first consolidated work on spiders of Himalayan region of Kashmir with detailed distributional data of spiders of this region.

OBJECTIVES

1. Survey and collection of spiders from Himalayan region of Kashmir
2. Identification of collected spiders from Himalayan region of Kashmir
3. Molecular identification of important spider species through DNA barcoding
4. Description of new species (if any) and re-description of important known species of spiders from Himalayan region of Kashmir
5. Devise keys to genera and species of spiders of Himalayan region of Kashmir

METHODOLOGY

A. Collection area:

Spiders will be collected by random sampling from all the districts of Kashmir viz. Anantnag, Bandipora, Baramulla, Budgam, Ganderbal, Kulgam, Kupwara, Pulwama, Shopian and Srinagar.

B. Collection of spiders:

In accordance with different standard and widely accepted methods spiders will be collected during the proposed study. These include:

1. **Visual search method** (Sudhikumar *et al.,* 2005): In this method plant parts and litter of the sampling areas are checked visually to look for spiders which is followed by their collection.
2. **Sweeping net method** (Miliczky *et al*., 2000): This method employs the use of a deep net attached to a long sizable handle. It is swept across foliage back and forth to collect spiders from plants.
3. **Stopper method**: This method is used to collect large slow-moving spiders. Open end of a bottle is placed near the spider and the spider is pushed carefully inside the bottle using the stopper.
4. **Beating tray method** (Chant, 1956): A beating tray consists of a cloth attached on a wooden frame. The tray is kept below a plant, this plant is then shaken or beaten with some stick so that the spiders fall on the cloth and are collected.
5. **Pitfall trap method** (Grbic *et al*., 2015): Ground dwelling spiders are collected by using this method. In this method pits are dug in the ground; the pits are fitted with some vessels which are filled with alcohol. Ground dwelling spiders fall in these traps and are collected.
6. **Aspirator method or Pooter method** (Leather, 2015): This method is used to collect minute spiders. Two straws of different diameters are used. The straw with a smaller diameter is fitted with gauze or filter paper at one end. This straw is then fitted in straw with larger

diameter. The open smaller end is placed near the spider to be collected and the larger open end is used to suck air. In this way the spider is collected on the gauze and is later blown out and preserved. Pooter apparatus with two tubes fitted in the cap of vial can also be used.

C. Preservation:

For morphological identification the collected spiders are preserved in 70 - 80 % alcohol, depending on the size of spider in glass tubes or collection vials with stoppers or eppendorf tubes. For molecular identification the spiders are preserved in 90% ethanol at -20^{0}C (Tyagi *et al*., 2019).

D. Labelling:

The vials containing spiders are labelled. Each label contains the information: date of collection, location from where the sample is collected along with the elevation and geo-coordinates using GPS system, type of web and habitat description.

E. Morphological Identification:

For identification, the preserved specimens are dried for studying morphological characters. Type of web also helps in identifying spiders. Spider genitalia are examined as per Herbert (1965) as genitalia play important role in their identification. After collecting the required information, the spiders are identified using available literature, various keys and catalogs provided by Platnick (2011), Ghavami, (2007), Barrion & Litsinger (1995), Davies & Zabka (1989), Tikader (1987), Kaston (1978), Locket & Millidge (1951) and other National and International literature.

F. Molecular identification:

DNA barcoding will be performed for molecular identification. It involves following steps:

1. **DNA isolation**: Spider leg is removed and DNA is isolated from it either manually/ by using Nucleospin Insect DNA kit (Macheray Nagel, Germany) or QIAamp DNA Investigator Kit (Qiagen, Valencia, CA).
2. **Gel electrophorsis for DNA confirmation**: Agarose gel electrophoresis is done for confirmation of extracted DNA in the sample as per protocol (Birren & Lai, 2012).
3. **Amplification of marker gene by PCR**: DNA barcode region of mtCOI (mitochondrial cytochrome oxidase subunit I) gene will be amplified using the primer pairs: LCO1490 and Chelicerate Reverse 1; LCO1490 and Chelicerate Reverse 2 (Barrett & Hebert, 2005) or any other suitable primer.

4. **Gel electrophoresis for confirmation of PCR products**: Agarose gel electrophoresis is done for confirmation of PCR products.
5. **Sequencing**: DNA Sequencing will be outsourced.

The data will then be analysed using BLASTn (Basic Local Alignment Sequence Tool) and BOLD (Barcode of Life Data System) and all the sequences will be uploaded to GenBank.

EXPECTED OUTCOME

The proposed Ph.D. research work will lead to the following results:

1. **New records and new species**: There is a great scope of collection and identification of new records and new species from Himalayan region of Kashmir so the present study will bring the consolidated record of spider species from this region.
2. **Description of new species (if any) and Re-description of important known species of spiders**: Description of new species (if any) and detailed re-description of important known species from Himalayan region of Kashmir will be carried out which will be helpful in correct identification of spider species in future.
3. **Preparation of keys to genera and species of spiders from Himalayan region of Kashmir**: Keys to genera and species will be prepared which will be helpful in future studies of spiders.
4. **Consolidated work on spiders from Himalayan region of Kashmir**: This will be the first consolidated work on spiders from Himalayan region of Kashmir.
5. **DNA barcodes for spiders from Himalayan region of Kashmir will be generated**: The DNA barcodes will help in the development of global molecular identification for spiders.

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